CLAIMS

1. A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, the method comprising the steps of:

(i) reverse transcription of the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each primer sequence is unique, and either or each heel sequence includes a RNA polymerase promoter site, and the FAP includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence; and

(ii) amplification of the cDNA using primers sufficiently complementary to the primers, i.e. FAP and TAP, within FAP-RAND and TAP-RT.

2. A method according to claim 1, which additionally comprises the step of:

(iii) *in vitro* transcription, to produce RNA run-offs from either end of the amplicons.

3. A method according to claim 1 or claim 2, wherein each heel sequence includes a different RNA polymerase site.

4. A method according to claim 3, for the production of a strand-specific library.

5. A method according to any preceding claim, for the production of a subtracted library from two cell populations.

6. A method according to any preceding claim, which comprises cloning the polynucleotide products and immobilising them in an array.

7. A method according to any preceding claim, wherein the sample is from laser capture microdissection.

8. A method according to any preceding claim, wherein the sample is from patch clamp harvesting.

9. A method according to any preceding claim, wherein the first and/or the second heel sequence includes the nucleotide sequence of a cleavage site.

10. A method according to claim 9, wherein the cleavage site is located at the 3' end of its heel sequence.

11. A method according to claim 10, wherein the first and second heeled primers have identical cleavage sites.

12. A method according to claim 10, wherein the first and second heeled primers have different cleavage sites.

13. A method according to any of claims 9 to 12, which comprises the additional step of treating the polynucleotides with an agent that cleaves at the cleavage site.

14. A method according to any preceding claim, wherein amplification comprises up to 50 amplification cycles.

15. A method according to claim 14, wherein each amplification cycle comprises the steps of :

(i) obtaining single-stranded DNA molecules at between 85°C and 97°C;

(ii) annealing the single-stranded DNA molecules at between 45°C and 65°C; and

(iii) elongating the annealed DNA molecules at between 70°C and 75°C .

16. A method according to any preceding claim, wherein the first heeled primer population consists of a population of nucleic acids comprising, from 5' end to 3' end :

(i) a heel sequence, of 15 to 22 nucleotides, which is not complementary to the mRNA molecules initially present in the sample; and

(ii) an oligo dT sequence of 15 to 25 nucleotides;

wherein substantially every possible variable sequence combination is found in said first heeled primer population.

17. A method according to any preceding claim, which additionally comprises confirming the presence of at least one nucleic acid sequence contained in the reaction mixture after amplification.

18. A method according to claim 17, wherein the confirming comprises any one of:

(i) detection of sequences of interest with specific oligonucleotide probes;

(ii) amplification of sequences of interest with specific oligonucleotide primers; and

(iii) cloning of the DNA molecules obtained in a replication and/or expression vector.